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RESEARCH ARTICLE

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Fat, meat quality and sensory attributes of Large White × Landrace barrows fed with crude glycerine

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Abstract

The use of alternative raw materials like crude glycerine in animal feed to reduce final costs could be of interest as the sector seeks to increase its competitiveness. The aims of the present work were to evaluate the effect of crude glycerine on back-fat thickness and the proximate composition of pork and to examine the effect on pork quality of using growing-finishing feeds with different percentages of crude glycerine added. For this purpose 60 crossbreed (Large White × Landrace) barrows were subdivided into three groups according to the crude glycerine concentration administered in feed: C, control diet, no crude glycerine; and G2.5 and G5 with 2.5% and 5% added crude glycerine, respectively. This study evaluated proximate composition, pH, cooking losses, texture, colour coordinates, fatty acid profile, and sensorial analysis. No differences were found in any of the three groups studied (C, G2.5, G5) for measurements performed both before (with ultrasound equipment) and after slaughter (millimetre ruler). The proximate composition and the physical-chemical parameters of *longissimus dorsi* were similar between groups. There were no differences detected ($p > 0.05$) between the three groups as regards the CIELab coordinates, textural profile and sensory attributes. Therefore, 5% crude glycerine to replace corn could be used as an ingredient in pig feed without appreciably affecting the back-fat and meat quality characteristics.

Additional key words: pig; pork quality; glycerol; by-product; stearic acid.

Introduction

In recent years the decreased availability of the raw materials traditionally used in feed production (cereals, soybean, etc) and their high prices have had a substantial impact on the animal production sector where feeding accounts for about 70% of total costs (Tible *et al.*, 2007). It is for this reason that the use of alternative raw materials in animal feed to reduce final costs could be of interest as the sector seeks to increase its competitiveness. Among such alternatives, crude glycerine, a by-product of biodiesel production, might be considered a useful source of energy. The global annual biodiesel production is projected to be about 41 billion litres in 2019 according to report by the OECD-FAO (2012). Furthermore it seems that biodiesel production capacities are growing all over the world (Kovacs *et al.*, 2011). For example, the European Union produced 5,140 million liters in 2005 and its production capacity was close to 10,850 million litters in 2012 (USDA, 2012). Biodiesel can be produced from different seeds (*e.g.* rapeseed, soybean, sunflowers, canola), palm oil, frying oils and fat. The esterification process produces glycerol as subproduct. One tonne of biodiesel gives about 100 kg of crude glycerine, which can be used as an energy source under oxidizing conditions since one mol glycerol yields 22 moles of ATP. Despite the fact that this by-product is extensively used in the food, cosmetic and pharmaceutical industries, its generalised use is limited due

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Abbreviations used: AI (aroma intensity); ATP (adenosine triphosphate); BFT (back-fat thickness); C (control); CL (cooking losses); CHE (chewiness); DFD (dark firmness and dry FI (flavour intensity); FIB (fibrousnesses); G2.5 (2.5% glycerol); G5 (5% glycerol); JUI (juiciness); LT (loin thickness); MC (meat colour); ME (metabolizable energy); OO (off-odour); PER (persistence); TEN (tenderness); TPA (texture profile analysis); WHC (water holding capacity).

to the possible presence of impurities (*e.g.* methanol, salts, fatty acids), so that the development of other uses, as in animal feed, could be important for the sustainability of biodiesel industries (Nitayavardhana & Khanal, 2011). In this respect some authors (Alexandre *et al.*, 2012; Quispe *et al.*, 2013) have confirmed the economic advantages of using crude glycerine, obtained from biodiesel, as a component of animal feed, since any reduction in feeding costs should lead to a reduction in meat and meat product prices.

Glycerol is a sweet-tasting product and so it is well-accepted by animals (Kerr *et al.*, 2007). In addition, it can be converted into glucose in the liver and provide energy through the gluconeogenic pathway. It also has osmotic properties that can affect muscle quality (Parker *et al.*, 2007). While some authors (Cerneau *et al.*, 1994; Mourot *et al.*, 1994; Airhart *et al.*, 2002; Hanczakowska *et al.*, 2010) reported that meat from pigs fed with crude glycerine showed some changes in their quality characteristics, others (Kijora & Kupsch, 1996; Hansen *et al.*, 2009; Mendoza *et al.*, 2010) did not describe any effect of this compound on pork quality. Many of these studies were carried out to ascertain the viability of using crude glycerine in pig nutrition, but few have focused on the effect of crude glycerine on pork quality aspects such as its sensory or textural profile. Due to the great variability in results concerning pork quality when a given percentage of crude glycerine is included in the feed formulation (Schieck *et al.*, 2010) or drinking water (Della Casa *et al.*, 2009) and the potential economic profits of using this renewable by-product as a substitute for more expensive raw materials, further research in this area is required.

The objectives of the present work were: (1) to evaluate the effect of crude glycerine on back-fat thickness and the proximate composition of pork and (2) to examine the effect on pork quality (pH, water losses, textural profile, colour, fatty acid profile and sensorial attributes) of using growing-finishing feeds with different percentages of crude glycerine added.

Material and methods

Animals and diets

All procedures involving animals were approved by the Ethics Committee of the University of Murcia, according to EU Directive 86/609 (OJEU, 1986) as modified by Directive 2003/65 (OJEU, 2003), which regulates the welfare of animals used in research and for scientific purposes. Sixty castrated crossbreed males (Large White × Landrace) were blocked by initial body weight (BW) (30.7 ± 0.02 kg; 80 ± 3.5 days of age) and assigned to 12 pen (5 pigs per pen, 1.5 m^2 space per pig) to evaluate the crude glycerine inclusion (Abengoa Bioenergía San Roque Cádiz, Spain) in the pig's diet on fat and loin thickness (LT), proximal composition, pH, water holding capacity (WHC), cooking losses (CL), texture, colour and sensory quality. All piglets were castrated before weaning.

The trial lasted 82 days and was conducted from October to December. Three experimental treatments were established according to the crude glycerine concentrations administered in the growing and finishing feed: C, control diet (no crude glycerine), G2.5 and G5 with 2.5% and 5% of crude glycerine, respectively. Twenty animals per treatment were used (four pen per treatment). The maximum crude glycerine levels included (5%) were established considering the manufacture feed technology capacity. The crude glycerine partially replaced corn in the diets which were formulated to be isoenergetic. The digestible lysine and the metabolizable energy (ME) ratio was similar in all the diets, for each phase. The amino acid diets were formulated according to ideal protein concept according to the recommendations of FEDNA (2006). The composition of the diet and the crude glycerine is summarized in Table 1.

Grower diet was fed from 30.7 to 64.2 kg BW (80 to 125 days of age) and finisher diet from 64.2 to 97.3 kg BW (125 to 162 days of age). Feeds and water were provided *ad libitum*.

The day before slaughter, the back-fat thickness (BFT) was measured by ultrasound (Prosound 2) using a 172 mm linear probe applied between the 10th and 11th ribs; the image site was determined by palpation. In order to establish a correct contact between animal hide and transducer, the area was thoroughly clipped and oiled. The following measurements were made: BFT and LT.

Two batches of 30 animals (10 per treatment each one) were randomly selected to be slaughtered in two consecutive days. The transport from the farm to the slaughter-house was approximately half an hour (20 km located in Lorca, Murcia, Spain). Pigs were slaughtered according to Directive 1009/2009 (OJEU, 2009). The carcasses were chilled at 4°C for 24 h, and then *longissimus dorsi* muscle was obtained.

Table 1. Composition of the diets used in the experiment

Feed	Grower diet[a]			Finisher diet[b]		
	Crude glycerine, %			Crude glycerine, %		
	0	2.5	5	0	2.5	5
Ingredients (g/100 g as feed)						
Barley	30.8	30.8	30.5	38.5	38.1	37.4
Wheat	30.0	30.0	30.0	25.0	25.0	25.0
Soybean meal, 47% CP[c]	17.0	17.4	18.1	15.0	15.5	16.1
Corn	15.0	12.5	10.0	15.0	12.5	10.0
Lard	3.70	3.59	3.51	3.02	3.05	3.10
Crude glycerine[d]	—	2.50	5.00	—	2.50	5.00
Calcium carbonate	1.16	1.15	1.15	1.31	1.55	1.54
Monocalcium phosphate	0.46	0.46	0.46	0.49	0.49	0.49
Sodium bicarbonate	0.39	0.20	—	0.38	0.18	—
Sodium chloride	0.30	0.20	0.15	0.40	0.20	0.40
VTM premix[e]	1.10	1.10	0.11	0.90	0.90	0.90
Calculated composition (g/100 g as feed)[f]						
ME[g], MJ kg^{-1}	13.5	13.5	13.5	13.5	13.5	13.5
Dry matter	88.6	88.7	88.8	89.0	89.1	89.2
Ash	4.68	4.63	4.62	4.92	5.01	5.24
Ether extract	5.75	5.56	5.39	5.00	5.00	5.00
Neutral detergent fiber	11.7	11.5	11.3	12.0	11.8	11.5
Linoleic acid	1.24	1.18	1.13	1.14	1.10	1.06
Ileal digestible Lys, g kg^{-1}	0.95	0.95	0.95	0.79	0.79	0.79

[a] Grower diet: 80-125 d. [b] Finisher diet: 125-162 d. [c] CP: crude protein. [d] Chemical composition: total glycerol, 86.66%; methanol, 0.0038%; moisture, 7.50%; ash, 5.88%; chloride, 3.06 %; calcium, 0.0040%; sodium, 2.005%; potassium, 0.0526%. [e] VTM: vitamin and trace mineral; Provided (per kg of complete diet): 6.0 and 4.9 g of L-lysine 50, 0.9 and 0.2 g of L-threonine and 0.7 and 0.3 g of DL-methionine for growing and finishing feed, respectively; 8,000 IU of vitamin A; 1,100 IU of vitamine D3; 20 IU of vitamin E; 1 mg of vitamin K_3; 1 mg of vitamin B_1; 3 mg of vitamin B_2; 1 mg of vitamin B_6; 0.015 mg of vitamin B_{12}; 17 mg of niacin; 10 mg of pantothenic acid; 0,08 mg of biotin; 0.02 mg of folic acid; 50 mg of choline; 50 mg of Mn; 0.5 mg of I; 90 mg of Zn; 10 mg of Cu; 90 mg of Fe; and 0.3 mg of Se; 10 IU of endo-1,4-beta-xylanase (CE 3.2.1.8) from *Bacillus subtilis* (LMG s-15136). [f] According to FEDNA (2003). [g] ME: metabolizable energy.

Back-fat thickness

At 24 h post-mortem, the BFT was measured in the carcasses in three points using a millimetre ruler: BFT_1 (measured in the back, at the first rib), BFT_2 (measured between the 9th and 10th ribs) and BFT_3 (measured in the thinnest layer of the *gluteus medius* muscle).

Sampling and physico-chemical analyses

Instrumental meat quality was assessed in the *longissimus dorsi* muscle. Each loin was then cut into diffrent pieces to evaluate the following meat quality parameters:

— Proximate composition (moisture, total protein, intramuscular fat content) was assessed by AOAC (1990) procedures.

— The pH was measured using a portable Crison GLP21 equipment with a penetrating electrode (ISO 2917:1999). It was calibrated using two different potassium chloride standard (pH 4 and 7). The meat pH measurements were taken at 45 minutes (pH 45) and 24 hours (pH 24) post-mortem.

— Water-holding capacity (WHC) was expressed in percentage (Grau & Hamm, 1953). To determine CL, each sample (fillet 50 g weight, 20 mm thickness) was placed in polyethylene bag and cooked in a water bath at 75°C for 15 min until an internal temperature of 72°C. The differences in the weight of raw and cooked samples were used to calculate percentage of cooking losses (Honikel, 1998). These cooked meat samples were cut into 20 × 20 mm cubes, using a stainless steel cutter. A texture profile analysis (TPA) was made using a QTS-25 texture analyzer (Brookfield

CNS Farnell, Borehamwood, Hertfordshire, England) equipped with a load cell of 25 kg and Texture Pro V. 2.1 software. Two consecutive cycles of 50% compression, with the cross-head moved at a constant speed of 30 mm min^{-1} were carried out. Texture variables, hardness (expressed as N), cohesiveness (no units), springiness (expressed as mm), gumminess (expressed as N) and chewiness (expressed as N-mm) were calculated as described by Bourne (2002). Six measurements per sample were made.

— Colour measurements (*L*, a*, b** coordinates) were made using a Minolta CR400 colorimeter calibrated against a standard white tile. Measurements were taken on the surface of the *longissimus dorsi* (8-mm-diameter aperture, d/0 illumination system, D65 illuminant and a 2º standard observer). Chroma and hue value were calculated as $C^* = (a^2 + b^2)^{1/2}$ and H = (arctg b*/a*)*57.32.

— Fatty acid profile in intramuscular fat was analyzed according to Granados (2001). Methylated samples were injected in an Agilent 6890N Network GC System equipped with a flame ionisation detector and a phenyl-methyl-xyloxane capillary column (HP5), 30 m long and with an interior diameter of 0.32 mm and 0.25 m film thickness. The detector and injector were maintained at a temperature of 300°C and 280°C, respectively. Helium was used as carrier gas, at a flow of 3.2 mL min^{-1} and a division ratio of 1:50. The methyl esters of fatty acids were quantified using undecanoic acid methyl ester as an internal standard.

All these analyses mentioned above (pH, proximate composition, WHC, colour coordinates and CL) were evaluated by duplicate at 24 h post-mortem. For the rest of the meat quality parameters (protein content, TPA, fatty acid profile and sensory analysis) the samples were frozen at –18°C until further analysis.

Sensorial evaluation

For the sensory analysis the pork loins were thawed in a conventional chiller at 4°C overnight. For cooking, the samples were placed between two heating plates covered with aluminium foil (Silanos, Liscia Average, Lavastoviglie Industriali, Italy) at 150°C for 6 min to reach an internal temperature of 72°C, as measured by a portable T200 thermometer (Digitron Instrumentation Ltd., Merd Lane, Hertford, UK). Rectangular pieces of approximately 15 × 20 mm from the loin centre were obtained and covered instantly with aluminium foil. Samples were kept at 60°C in sand baths (Braun, Espluges de Llobregat, Barcelona, Spain) until they were presented to the panellists in a balanced order (Macfie *et al.*, 1989). The panel was formed of eight assessors chosen from the staff of the University of Murcia with ages ranging between 24 and 45 years; five women and three men; all experienced in the profile assessment of different meat products and trained according to ISO 8586-2 (2008). Seven training sessions were carried out: in the first three, descriptors of raw/cooked pork loins were studied and the following four sessions were concerned with identifying, selecting and quantifying attributes to evaluate the meat. In both the training and assessment sessions, the samples were coded with random three digit numbers. Mineral water was provided for rinsing between samples. Sensorial analysis was carried out according to ISO 4121 (2003) using an unstructured scale of 10 cm. The descriptors used were: aroma intensity (AI), off-odour (OO), flavour intensity (FI), persistence (PER), meat colour (MC), juiciness (JUI), tenderness (TEN), chewiness (CHE) and fibrousnesses (FIB).

The standard National Pork Producer Council (NPPC, 1991) scale was used to determine the marbling (1 minimal infiltration, up to 6, maximal infiltration).

Statistical analysis

Data were analyzed with the statistical package SPSS 15.0 (Statistical Package for the Social Science). Animal was used as experimental unit. The effect of the different dietary treatments on meat quality was analysed using an analysis of variance (ANOVA). When the differences among groups were significant ($p < 0.05$), Tukey's test at a significance level of $p < 0.05$ was carried out to evaluate the differences between the treatments. Pearson correlation coefficients were evaluated for the back-fat and LT.

Results

Back-fat and loin thickness

Table 2 shows that back-fat and LT measurements by ultrasound and with a millimeter ruler (BTF1, BF2

Table 2. Back-fat thickness (mean in mm) measured with millimetre ruler (BFT_1, BFT_2 and BFT_3) and ultrasound (BFT and LT) in pigs fed with 0, 2.5 or 5% of crude glycerine

Item[a]	Treatment[b]			SE[c]	Significance[d]
	Control	G2.5	G5		
n	20	20	20		
BFT_1	39.6	43.9	40.5	0.06	ns
BFT_2	26.4	26.8	26.2	0.05	ns
BFT_3	23.1	26.3	25.8	0.08	ns
BFT	18.2	19.2	19.0	0.57	ns
LT	41.6	39.7	41.9	0.84	ns

[a] Back-fat thickness: 1 (BFT_1), measured at the first rib with the millimetre ruler; 2 (BFT_2), measured between the 9th and 10th ribs with the millimetre ruler; 3 (BFT_3), measured in the *Gluteus medius* muscle, at the lowest fat thickness area with the millimetre ruler; BFT, measured from the first hyperecogenic layer (skin) to the hyperecogenic layer above muscle (hypoecogenic); Loin thickness (LT), measured from where the back-fat terminates to the bottom of the muscle (end of the hypoecogenic layer). [b] Control, G2.5, G5: pigs fed with 0%, 2.5%, 5% crude glycerine in diet, respectively. [c] SE: standard error of the mean. [d] ns: non-significant ($p > 0.05$).

and BTF 3). No differences were found in any of the three groups studied (C, G2.5, G5) for measurements performed both before (with ultrasound equipment) and after slaughter (millimetre ruler). Significant Pearson correlation between BFT with BFT_2 and BFT_3 were found ($R^2 = 0.487$ and 0.581; $p < 0.05$).

Meat quality

Proximate composition, pH and water content. Table 3 shows meat proximate composition, pH, WHC and CL from pigs fed different crude glycerine levels. These parameters did not vary by effect of crude glycerine inclusion in diets ($p > 0.05$).

Texture (textural profile analysis), colour coordinates (L, a* and b*) and marbling.* Meat texture results are shown in Table 4. The inclusion of crude glycerine in the feed did not have any effect ($p > 0.05$) on the meat texture parameters. Table 4 also shows the results of the objective instrumental evaluation of colour (L*, a*, b* and H * and C*). The coordinate means values were 49.85, 10.3 and –1.8 for L*, a* and b* respectively, with no treatment effects ($p > 0.05$).

Fatty acid profile. Table 5 shows the fatty acid profile of intramuscular fat obtained from the *longissimus dorsi* muscle. No significant differences were observed in the pork fatty acid composition when crude glycerine was added in growing and finishing feeds.

Table 3. Proximate composition (%) (mean), pH (at 45 minutes and 24 hours post-mortem), water holding capacity (WHC, % free water) and cooking losses (CL, %) in *longissumus dorsi* from pigs fed with 0, 2.5 or 5% of crude glycerine

Item	Treatment[a]			SE[b]	Significance[c]
	Control	G2.5	G5		
Fat	3.45	4.30	3.80	0.246	ns
Moisture	73.24	72.26	72.65	0.492	ns
Protein	23.00	22.86	22.74	0.882	ns
Ash	1.45	1.46	1.40	0.130	ns
pH 45	6.09	6.11	6.17	0.042	ns
pH 24	5.35	5.31	5.39	0.020	ns
WHC[d]	71.46	73.70	72.45	0.508	ns
Cooking loss	26.49	27.12	25.86	0.442	ns

[a] Control, G2.5, G5: pigs fed with 0%, 2.5%, 5% crude glycerine in diet, respectively. [b] SE: standard error of the mean. [c] ns: non-significant ($p > 0.05$). [d] WHC: water holding capacity.

Table 4. Texture profile analysis, colour coordinates (*L**, *a**, *b**, H*, C*) and marbling in *longissimus dorsi* from pigs fed with 0, 2.5 or 5% of crude glycerine

Item	Treatment[a]			SE[b]	Significance[c]
	Control	G2.5	G5		
Texture					
Gumminess	23.51	23.58	22.49	1.257	ns
Adhesiveness	–0.20	–0.27	–0.17	0.014	ns
Cohesiveness	0.55	0.54	0.56	0.005	ns
Chewiness	99.44	90.38	80.55	4.942	ns
Springiness	4.20	3.95	3.55	0.066	ns
Hardness	41.72	43.30	40.12	1.788	ns
Colour					
*L**	50.25	50.20	49.11	0.570	ns
*a**	10.51	9.97	10.38	0.166	ns
*b**	–1.73	–1.73	–2.01	0.120	ns
H*	–9.78	–10.32	–11.30	0.717	ns
C*	10.17	10.16	10.61	0.154	ns
Marbling[d]	1.22	1.28	1.16	0.053	ns

[a] Control, G2.5, G5: pigs fed with 0%, 2.5%, 5% crude glycerine in diet, respectively. [b] SE: standard error of the mean. [c] ns, non-significant ($p > 0.05$). [d] Standard National Pork Producer Council (1991).

Sensory analysis. Fig. 1 shows the sensory attributes of meat from pigs fed different crude glycerine levels. There were no differences among treatments groups for AI, OO, FI, PER, MC, JUI, TEN, CHE and FIB.

Discussion

Back-fat and loin thickness

In this work, crude glycerine had no effect on BFT, as confirm Lammers *et al.* (2008b), Hansen *et al.* (2009),

Table 5. Fatty acid composition (g/100 g) (mean) of intramuscular fat from pigs fed with 0, 2.5 or 5% of crude glycerine

Fatty acid[a]	Treatment[b]			SE[c]	Significance[d]
	Control	G2.5	G5		
10:0	0.09	0.09	0.10	0.003	ns
12:0	1.38	1.14	1.36	0.095	ns
14:0	1.30	1.41	1.34	0.023	ns
16:0	24.56	24.75	24.59	0.326	ns
16:1	2.08	2.16	2.26	0.052	ns
18:0	11.84	10.88	11.56	0.166	ns
18:1	40.45	41.06	41.16	0.338	ns
18:2	17.08	17.51	17.28	0.263	ns
18:3	0.16	0.08	0.10	0.027	ns
20:0	0.70	0.72	0.66	0.012	ns
SFA	38.78	36.13	39.43	0.631	ns
TUFA	61.22	63.87	60.58	0.631	ns
MUFA	43.98	46.28	43.19	0.736	ns
PUFA	17.24	17.59	17.38	0.263	ns

[a] SFA: saturated fatty acid; TUFA: total unsaturated fatty acid. MUFA: monounsaturated fatty acid; PUFA: polyunsaturated fatty acid. [b] Control, G2.5, G5: pigs fed with 0%, 2.5%, and 5% crude glycerine in diet, respectively. [c] SE: standard error of the mean. [d] ns, non-significant ($p > 0.05$).

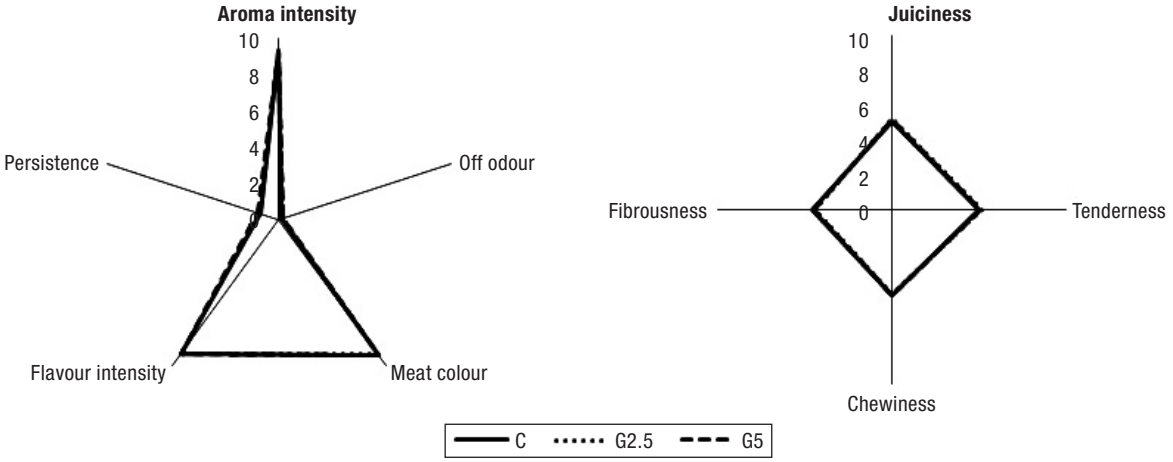

Figure 1. Sensory analysis (aroma intensity, off odour, flavour intensity, persistence, meat colour) and texture profile (juiciness, tenderness, chewiness and fibrousness) in *longissimus dorsi* from pigs fed with 0% (C, control), 2.5% (G2.5) or 5% (G5) of crude glycerine.

and Schieck *et al.* (2010), that found no significant differences in the meat from pigs fed with less than 15% crude glycerine added. In contrast, some authors like Hanczakowska *et al.* (2010) reported a decrease in adipose fat in animals (110 kg slaughter BW) fed with 10% crude crude glycerine (19.2 mm) compared with control pigs (21.6 mm) and those fed with 10% refined crude glycerine (20.9 mm). It is seems that more than 15% crude glycerine in the diet leads to a linear increase of fat depth at point BFT_2 (tenth costal vertebra) and a lower percentage of fat free lean (Stevens *et al.*, 2008).

Significant Pearson correlation between BFT with BFT_2 and BFT_3 may be suitable the validity of using real-time ultrasound in animals before slaughter to develop equations to predict the thickness of back-fat in the carcasses pigs as reported Velázquez (2000) for pigs body weighing between 80 and 120 kg.

Meat quality

Proximate composition, pH and water content

The mean values obtained were 3.8% fat, 72.7% moisture, 22.8% crude protein and 1.4% ash, which are similar to those reported by Della Casa *et al.* (2009) in Italian Duroc and Italian Large White cross heavy pigs fed with different crude glycerine percentages. Teixeira & Rodrigues (2013) found similar levels of fat in Large White and Landrace cross pigs, with a slaughter weight between 80 and 100 kg.

Crude glycerine did not affect pH of meat, which agrees with other researches (Della Casa *et al.*, 2009; Hansen *et al.*, 2009; Berenchtein *et al.*, 2010; Mendoza *et al.*, 2010; Schieck *et al.*, 2010). In contrasts, Lammers *et al.* (2008b) observed a slight trend for the loins of Cambrough 22 and L337 cross pigs fed 5% and 10% crude glycerine to have a higher final pH than the loins from control animals.

Similar results were found for WHC and CL in previous studies (Kijora & Kupsch, 1996; Lammers *et al.*, 2008b; Hansen *et al.*, 2009; Berenchtein *et al.*, 2010; Mendoza *et al.*, 2010; Schieck *et al.*, 2010). However, Mourot *et al.* (1993) emphasized a reduction in water losses and CL in Large White animals fed 5% crude glycerine from 30 to 100 kg of live weight. This was attributed to its action on cell osmotic pressure, what would increase the water content, and then, the WHC. The muscle fibers would be hyperhydrated, due to the increased water content of cells subjected to a lower degree of protein denaturation, especially during heat treatment. Similar results were described by Aihart *et al.* (2002), who found a trend towards a reduction in these parameters in meat from pigs fed glycerol. In addition, in a study with pigs fed 10% crude or refined glycerine, Hanczakowska *et al.* (2010) also found a higher WHC. The data regarding water losses in the literature vary substantially and Schieck *et al.* (2010) identified the NaCl content of feed as the main factor causing differences between groups. Sodium is often added as a catalyst in biodiesel production, which turns into NaCl after purification. The amount of NaCl in crude glycerine depends on the refining technique used, and should be taken into account when formulating diets (Kerr *et al.*, 2007). An increased intake of NaCl would result in a higher salt content in the mus-

cle, which would result an increased WHC of meat due to myofibril edema. In our experiment, the Na^+ and Cl^- content of the crude glycerine used in feed was considered and similar electrolytic balance was formulated in feed for each phase.

Texture (Textural Profile Analysis), colour coordinates (L, a* and b*) and marbling*

Glycerine addition seems no affect meat texture. Similar results were found by other authors as Duttlinger *et al.* (2008), Della Casa *et al.* (2009), Hansen *et al.* (2009) and Berenchtein *et al.* (2010) who evaluated the meat texture with a Warner-Bratzler cell (WB). Although different methodology was used (WB *vs.* TPA), Caine *et al.* (2003) found a positive correlation between the maximum force parameter obtained by the WB method and hardness (maximum force obtained during the first compression cycle) resulting from a TPA analysis. Others, including Gipe (2008), noted a tendency to greater hardness in the meat from pigs fed 2.5% crude glycerine than meat from a control group and a group fed 5% crude glycerine, which was attributed to the more apparent connective tissue in the meat samples. In the present study the absence of significant differences in texture parameters between the 2.5% and 5% crude glycerine groups suggests that about 5% crude glycerine could be included in pig diet without affecting meat tenderness.

There were no differences between treatments for the CIELab coordinates nor for the C* and H*, which agrees with the results obtained by other authors (Della Casa *et al.*, 2009; Hanczakowska *et al.*, 2010; Schieck *et al.*, 2010). These values could be affected by numerous intrinsic and extrinsic factors that affect meat colour (Sañudo *et al.*, 1998). Marbling, based on a subjective standard scale (NPPC, 1991), showed no significant differences between the different groups, as reported by Gipe (2008), Mendoza *et al.* (2010) and Schieck *et al.* (2010), in meat of pig fed glycerine.

Fatty acid profile

The fatty acid profile of intramuscular fat obtained from the *longissimus dorsi* muscle was not affected when crude glycerine was added in growing and finishing feeds. These results agree with the reports of other authors in pigs fed glycerine (Cerneau *et al.*, 1994; Mourot *et al.*, 1994; Kijora *et al.*, 1997; Lammers *et al.*, 2008c; Della Casa *et al.*, 2009).

Other authors have found effects of glycerine in feeds on fatty acid profile. Thus, linoleic acid (18:2) remained unchanged by the effect of crude glycerine addition, in contrast to the findings of Mourot *et al.* (1993), Cerneau *et al.* (1994), Kijora *et al.* (1997), Lammers *et al.* (2008b) and Della Casa *et al.* (2009), who found this fatty acid to be reduced in pigs fed crude glycerine. Mourot *et al.* (1993) suggested that this change in the fatty acid profile could be related to a lower consumption of corn in the pig diet, which is rich in linoleic acid (FEDNA, 2003). In our study, the linoleic acid content in diet was also reduced by glycerol incorporation but differences between dietary treatments were very low (Table 1). The fact that linoleic acid is the most variable fatty acid as regards its concentration in meat is perhaps due to its being an essential fatty acid that cannot be synthesized *de novo* by animals, so that it must be taken from the diet (Granados, 2001). In addition, since fat composition closely depends on the fatty acid profile of the diet, the feed ingredients added will determine the fat quality, glycerol is an important structural component of triglycerides and phospholipids that can act as an intermediate in the lipogenesis pathway and yield energy through the citric acid cycle (Duttlinger *et al.*, 2012). On the other hand, crude glycerine could also been source of fatty acid *per se*, as previous studies found that crude glycerine could contain 56% of fatty acids, mainly unsaturated (Chiloane *et al.,* 2013). So the meat fatty acid profile could be influenced by a remainder of fatty acid in crude glycerine. In the present study, the crude glycerine fat content was negligible.

Sensory analysis

Crude glycerine included in feed had no effect on the sensory quality of pork. These results agree with those observed by Lammers *et al.* (2008a) and Schieck *et al.* (2010). In adittion, Hanczakowska *et al.* (2010) found no significant differences in tenderness or juiciness, but a significant decrease in odour and taste-related parameters in pigs fed crude crude glycerine, that were attributed to the effect of certain substances present in the crude preparation rather than glycerol per se.

In conclusion, the crude glycerine from the elaboration of biodiesel can be used at up to 5% in growing-

finishing feed for pigs to replace corn without affecting pork meat quality, including the sensory characteristics of cooked pork.

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